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Van Buskirk, Josh ; Krügel, Andri ; Kunz, Julia ; Miss, Fabia ; Stamm, Alba

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The Rate of Degradation of Chemical Cues Indicating Predation Risk: An Experiment and Review

Josh Van Buskirk, Andri Krügel, Julia Kunz, Fabia Miss & Alba Stamm

Evolutionary Biology & Environmental Studies, University of Zürich, Zürich, Switzerland

Correspondence
Josh Van Buskirk, Institute of Evolutionary Biology & Environmental Studies, University of Zurich, CH-8057 Zurich, Switzerland.
E-mail: josh.vanbuskirk@ieu.uzh.ch

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Abstract

Many prey taxa use kairomones or alarm pheromones to assess the risk of predation in aquatic environments, and the rate at which these cues attenuate determines how precisely they indicate the local density of predators. We estimated the rate of degradation of chemical cues generated by *Aeshna* dragonfly larvae feeding on *Rana temporaria* tadpoles. The half-life of the cue was 35 h and was not influenced by whether it was aged in pond water or tap water or whether other tadpoles were present in the container in which cue-aging occurred. A review of other published estimates of predator cue half-life revealed values of 0.2–126 h, and variation among studies was unrelated to the type of aging water, the venue in which water was aged or prey behavior observed (laboratory, field), or the type of behavior that was recorded. We conclude that factors affecting the persistence of predator cues remain uncertain in spite of their importance for understanding the evolution of induced defenses.

Introduction

Many animals use chemical cues to assess the local risk of predation (Kats & Dill 1998). In aquatic systems, chemical cues are the most important manner by which prey detect predators (Dodson et al. 1994; Chivers & Smith 1998; Hettyey et al. 2010). Little is known about the chemical structure of water-borne chemical cues; available evidence suggests that these cues differ widely even among similar taxa (Brown et al. 2003; Lass & Spaak 2003; Zimmer et al. 2006). However, we have good evidence that they can originate from both predators and prey, and they contain remarkably specific information about the intensity and kind of risk in the environment (Van Buskirk & Arioli 2002; Smith et al. 2008; Schoeppner & Relyea 2009).

The special properties of chemical cues may influence the relationship between prey and predators in aquatic systems. Water-borne chemical cues take longer to disperse through the environment than other modalities such as visual or auditory cues, but they also persist longer and may travel farther under visually obstructed conditions. Depending on the temporal and spatial scales of predator occurrence and cue activity, chemical cues might contain more or less information than direct cues. If risk fluctuates rapidly relative to cue persistence and cues disperse long distances relative to spatial variation in risk, then chemical cues will contain only limited information about the current location of predators (Turner & Montgomery 2003; Fraker 2009; Chivers et al. 2013). But chemical cues could be highly predictive of predation risk if predator density varies little in space relative to cue dispersal and changes slowly in time relative to cue persistence.

The value of water-borne chemical cues to prey animals therefore depends on two empirical issues: What are the temporal and spatial scales of variation in predation risk? and what is the rate at which chemical cues deteriorate? This paper addresses the second issue. Previous studies show that cue concentration declines for two reasons: dilution and dispersal of the cue by turbulent flow, and degradation of the cue by natural processes. The first of these implies that conditions of high water flow or turbulence make water-borne predator cues less reliable and likely to induce weaker reactions (Large et al. 2011; and references

therein). The second process has been measured in several experiments on cue degradation, but the estimated rates of breakdown range over >2.5 orders of magnitude (Loose et al. 1993; Chivers et al. 2013). Even multiple studies of very similar predator–prey systems have yielded divergent estimates of the half-life of the cue, ranging from a few hours to several days (Peacor 2006; Ferrari et al. 2008; Fraker 2009). One explanation is that longer estimates occur when biodegradation is inhibited by artificial experimental conditions (Peacor 2006; Ferrari et al. 2008; Chivers et al. 2013).

Here, we describe an experiment in which biodegradation was indirectly manipulated in two ways to test the importance of biotic contributions to breakdown of predator cue. We also combine our new estimates of the cue deterioration rate with those of earlier studies to produce a quantitative appraisal of the causes of variation in degradation rate. Our approach was similar to those employed in earlier studies of predator cue degradation: We exposed naïve prey individuals to water containing predator cues of different ages and used the behavioral response of the assay prey as an indication of cue concentration. In our study, the prey were tadpoles of the frog *Rana temporaria,* and the predators were dragonfly larvae. We manipulated two factors in addition to chemical cues. The type of water in which cue was aged was either tap water or pond water, because the breakdown of chemical cues may be enhanced by the microbial degradation and adsorption onto organic matter that occur more readily in natural pond water (Peacor 2006; Ferrari et al. 2008). The presence or absence of tadpoles in the water during the aging process was manipulated because tadpoles themselves may contribute to cue breakdown by filtering particles from the water or by inoculation with microbes. Indeed, the response to predator cues is known to decline at high prey density (Hossie & Murray 2011; Van Buskirk et al. 2011). The mechanism underlying this pattern is proposed to involve density-dependent risk assessment (Peacor 2003), but our experiment tests whether cue decomposition depends on prey density as well.

Methods

The experiment had a 2 × 2 × 2 complete factorial design, with presence or absence of predator cues crossed with the type of water in which the cue was stored (tap water or pond water) and the presence or absence of tadpoles in the cue storage containers. Chemical cues were produced on six independent repetitions over a period of three weeks, and for each repetition, we assessed cue impact on the behavior of naïve assay tadpoles at seven time points (when cue was between 1 h and 72 h old). Cue production, storage, and behavioral observations of assay tadpoles were performed in an unheated laboratory room with open windows, with some natural lighting augmented by artificial lighting (16:8 light:dark schedule) and temperature between 18 and 21°C.

Cue Production

Chemical cues indicating predation risk were generated and aged in eight 5-l opaque plastic storage containers. Eight dragonfly larvae (*Aeshna cyanea*, instars F-0 and F-1) were each fed three *R. temporaria* tadpoles (200 mg tadpole mass per dragonfly) in 200-ml plastic cups filled with tap water (pH 7.7, nitrate 3.6 mg/l, phosphate <0.005 mg/l, hardness 16.5 °fH; www.stadt-zuerich.ch). The dragonflies consumed the tadpoles within about 15 min, after which the water from the feeding cups was mixed and then distributed into four of the storage containers (0.4 l each). The other four containers received 0.4 l tap water with no predator cues. We then filled the eight storage containers to 1.8 l total volume; depending on the treatment, the additional water was either tap water or drawn from an outdoor artificial pond. Half the storage containers received five *R. temporaria* tadpoles, the same size as the assay tadpoles described below. Tadpoles were fed rabbit food during the cue-aging process, at about 10% of their mass per day. This procedure produced the eight treatments of the factorial design. The containers remained on a laboratory shelf for 72 h, until the final behavioral assay was complete.

Behavioral Assays

We used the behavioral response of naïve assay tadpoles, measured at intervals of 1, 3, 6, 13, 24, 48, and 72 h after the dragonflies consumed their prey, to judge the persistence of chemical cues indicating predation risk. At each time interval, two tadpoles were placed into each of 32 opaque plastic bins (20 × 11 cm) filled with 0.5 l aged tap water and placed on a single laboratory shelf. After a 10-min acclimation period, we added 50 ml cue water to each bin from one of the cue storage containers (four bins for each type of water). We waited 5 min after adding the cue water and then observed the behavior of tadpoles in each bin on two visits lasting 10 s each, made by two different observers and

completed within a 5-min period. On each visit, we scored for each tadpole whether it remained immobile (score = 0), was moving <5 s (1), or was moving >5 s (2). Movement included feeding and swimming behavior. Averaging over both visits and both tadpoles, this generated an index of tadpole activity for each bin with nine possible values ranging from 0 (neither tadpole moved during either visit) to 2 (both tadpoles were moving more than half the time on both visits).

This entire procedure was repeated twice a week for three weeks. We disassociated time of day from cue age by initiating three repetitions at 07:00 and the other three at 17:30. Cue production and assessment of the eight treatments therefore occurred on six independent repetitions, and for each repetition, behavior was observed at seven time points. This yielded a large sample size (2688 assay tadpoles in 1344 bins), but true statistical independence occurred only at the level of the six repetitions.

The assay tadpoles came from five clutches collected near Zurich, Switzerland; eggs and hatchling tadpoles were held outdoors in 80-l pools and fed rabbit food *ad libitum*. The assay tadpoles grew somewhat larger over the three weeks of the experiment (mean mass ± SD; week 1: 132 mg ± 58, week 2: 137 mg ± 17, week 3: 184 mg ± 30). Each tadpole participated in the experiment only once.

Statistical Analysis

We analyzed the difference in activity between bins exposed to water with and without cue, calculated at the level of the four replicates within each repetition. This difference was negative if tadpoles reduced activity when they detected predators, which is generally the case (Skelly & Werner 1990; Van Buskirk 2002a). We began with a preliminary analysis to compare linear and negative exponential relationships between the activity difference and time. The linear model was better supported and was therefore used for hypothesis testing (AIC values: linear 1050.4, negative exponential 1059.7). We then tested the significance of water type, presence of tadpoles, and their interaction using a linear mixed-effects repeated-measures model with repetition serving as a random subject. The repeated measures were made through time within repetitions. Likelihood ratio tests comparing nested models with alternative random structures showed that variance among repetitions was very important (LR statistic = 61.0, df = 1, $p < 0.0001$). Heterogeneity in slopes on time among repetitions, the covariance between slopes and intercepts of repetitions, and replicates nested within repetitions were not important (all $p > 0.1$). The significance of fixed effects was assessed by inspecting 95% profile-likelihood confidence intervals of the parameters (Cox & Hinkley 1974, p. 343; Venzon & Moolgavkar 1988). A Q–Q plot confirmed that the residuals were close to normally distributed. Model R^2 was calculated as the variance explained by both fixed and random factors in the full model (Nakagawa & Schielzeth 2013). These analyses were implemented in the LME4 package in R 3.15.2 (Baayen et al. 2008; R Core Team 2013).

Results

Water-borne predator cues induced a strong behavioral response, which declined approximately linearly over time and disappeared completely by 72 h (Fig. 1). Average assay tadpole activity when exposed to fresh predator cue was about half that in the control treatment, but activity increased as the cue aged (Fig. 1a). Repeated-measures analysis of the difference between cue and control treatments indicated that the effect of fresh cue was −0.49 activity units (intercept of the model) and the rate of change was 0.16 units per day (coefficient for time; Table 1). The half-life of the cue was 36.5 h, estimated from the linear model. The type of water in which the cue was stored (pond water, tap water), and the presence of tadpoles in the storage container had essentially no influence on the behavioral response or the rate at which it changed through time (Table 1, Fig. 1b).

Discussion

This study of a predator–prey system involving dragonflies and tadpoles confirmed that chemical cues released by the predator and/or prey during a predation event transmit information about predation risk to prey and that the behavioral response induced by the cue declines as the cue ages. In this and other similar experiments on aquatic animals, the change in behavioral response is taken to reflect chemical degradation of the cue substance. However, experiments published so far report highly divergent estimates of the rate at which the response to cue declines with cue age. In 12 studies of 14 prey species that we located, values for the half-life of the predator cue range from 12 min to 45 h (Table 2).

What accounts for variation in the estimated rate of cue degradation? It has been suggested that deactivation proceeds faster when cues are exposed to microbial sources of degradation. This has been demonstrated in the laboratory for kairomones that affect

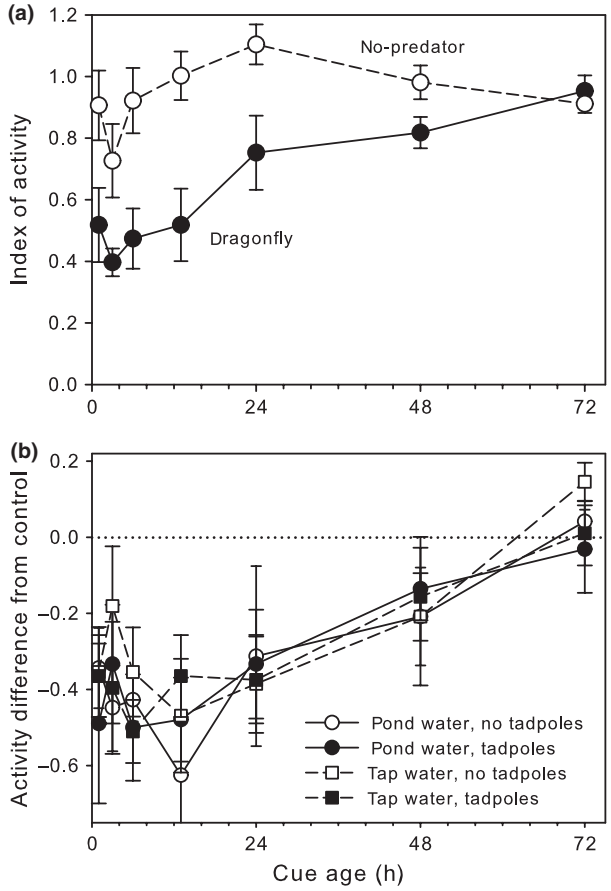

Fig. 1: Activity of assay tadpoles exposed to control water or water containing predator cues of different age (a) and temporal change in the activity difference between predator cue and control (b). Symbols represent mean ± 1 SE (n = 6 repetitions). The activity index ranges from 0 (tadpoles never active) to 2 (both tadpoles active more than half the time). In a, data were pooled across the non-significant effects of water type and tadpole presence. In b, the four cue-aging treatments are indicated by line type (pond water, tap water) and symbol shading (tadpoles, no tadpoles).

Daphnia behavior (Beklioglu et al. 2006), and microbes were indirectly implicated by Peacor's (2006) observation that the response of anuran prey declined more quickly when cue was stored in pond water rather than well water (half-life of 9–14 h vs. >40 h; Table 2). This finding was not confirmed in our study. It has also been proposed that exposure to sunlight hastens the rate at which chemical cues age (Ferrari et al. 2008); degradation can be faster at mid-day than in morning or evening (Chivers et al. 2013), and experimental exposure of *Chaoborus* cue to 5–10 h of ultraviolet radiation caused a 50% reduction in its effectiveness with *Daphnia* (Sterr & Sommaruga 2008). Persistence of the cue could also depend on whether it is produced by the prey, the predator, or both (Wisenden et al. 2009). Alarm pheromones, prey metabolites, constituents of digested prey, and predator digestive fluids may all function as signals of predation risk (Lass & Spaak 2003; Schoeppner & Relyea 2009), and these materials might differ in their reaction to sunlight or susceptibility to biodegradation. Finally, different kinds of behavioral response could show different patterns of change with cue age. Fresh cues probably trigger the full range of response, including microhabitat shifts and decreased activity, whereas older signals may be less likely to induce spatial avoidance because they carry less reliable information about the location of the predator. According to this explanation, variation in cue persistence among studies in Table 2 need not reflect variation in degradation rate, but could arise from differences in the behavioral response to chemical signals of different ages. All experiments so far would be vulnerable to this misinterpretation, because they all use prey behavior as an indirect indication of cue concentration.

Published results do not support any of these explanations. We analyzed estimates of half-life of the cue from the available data (Table 2, Fig. 2) using linear models, including study as a random effect and the total duration of the study as a covariate. In separate analyses, exposure to outdoor sunlight during the aging process, the type of behavioral response (activity/spatial location), and the behavioral assay venue

Table 1: Repeated-measures analysis on the difference in activity between tadpoles exposed to predator cues and no predator cues. Six independent repetitions, with four replicates of each treatment within each repetition, were observed repeatedly through time on seven occasions up to 72 h. The table shows estimated coefficients, the level at which the coefficient is estimated (for categorical effects), and profile confidence intervals; parameter estimates other than the intercept are multiplied by 100. Only the intercept and the change through time differ significantly from zero (indicated by bold text). Repetitions served as the random effect. Model R^2 was 0.092

Source	Level	Estimate	95% CI
Intercept	–	**−0.490**	**−0.605–−0.375**
Within-subjects effects			
Time in hours	–	**0.672**	**0.349–0.996**
Time × Water	Tap	−0.095	−0.552–0.361
Time × Tadpoles	Present	−0.033	−0.490–0.422
Time × Water × Tadpoles	Tap, present	0.069	−0.575–0.714
Between-subjects effects			
Water	Tap	9.740	−5.943–25.414
Tadpoles	Present	0.891	−14.748–16.529
Water × Tadpoles	Tap, present	−7.038	−29.177–15.112

Table 2: Results of studies estimating the persistence of a water-borne chemical cue indicating predation risk. The design features listed here include the predator taxon, the source of chemical cues (damaged prey, unfed predator, or predator consuming prey), the venue in which cues were allowed to age (indoors or outdoors and exposed to sunlight), type of water in which cues were aged ('Lab' is tap or well water, 'Natural' is pond water or seawater), the venue in which the behavioral response of the prey was assayed, the kind of trait that was used to gauge the response (change in activity or change in spatial location or microhabitat), and the duration over which the response was measured. Outdoor artificial ponds are here considered a field venue because they contain a range of microbes and are exposed to natural sunlight, photoperiod, and temperature. Estimated half-life is ln(2)/λ, where λ is estimated from the equation $y \sim c \exp(-\lambda \text{ time})$; y is the behavioral difference from the control, c is a constant, and time is the number of hours as the cue was produced. This model was fit by nonlinear least squares to data reported in figures and tables. A linear model was used for studies with only two time points. The first seven prey taxa are invertebrates, the next three are fish, and the last four are amphibians

Prey taxon	Predator taxon	Cue source	Aging venue	Aging water	Assay venue	Response trait	Duration of experiment	Estimated half-life (h)	Source
Oronectes virilis	Turtle	Predator	Indoor	Lab	Lab	Activity	2	1.87	Hazlett 1999
Procambarus clarkii	None	Prey	Indoor	Lab	Lab	Activity	24	14.4	Acquistapace et al. 2005
				Lab	Lab	Location	24	13.1	
Callinectes sapidus	None	Prey	Outdoor	Natural	Field	Location	36	13.5	Ferner et al. 2005
Daphnia magna	Fish	Both	Indoor	Lab	Lab	Location	24	126, 92[a]	Loose et al. 1993
				Natural	Lab	Location	24	126, 30	
Gammarus lacustris	None	Prey	Indoor	Lab	Lab	Activity	6	3.26	Wisenden et al. 2009
				Lab	Lab	Location	6	0.71	
Chaoborus flavicans	Fish	Predator	Indoor	Lab	Lab	Location	120	45.3	Oda & Hanazato 2008
Physa acuta	Fish	Both	Outdoor	Natural	Field	Location	96	41	Turner & Montgomery 2003
Pomacentrus amboinensis	None	Prey	Outdoor	Natural	Lab	Location	0.5	0.20	Chivers et al. 2013
Pimephales promelas	None	Prey	Indoor	Lab	Lab	Activity	6	3.42	Wisenden et al. 2009
				Lab	Lab	Location	6	5.56	
				Lab	Field	Location	6	∞[b]	
Phoxinus eos	None	Prey	Indoor	Lab	Field	Location	6	7.12	Wisenden et al. 2009
Rana catesbeiana	Dragonfly	Both	Indoor	Lab	Lab	Activity	100	44.2	Peacor 2006
				Lab	Lab	Location	100	40.1	
				Natural	Lab	Activity	100	13.9	
				Natural	Lab	Location	100	8.82	
Rana sylvatica	None	Prey	Outdoor	Natural	Lab	Activity	8	2.88	Ferrari et al. 2008
Rana clamitans	Dragonfly	Both	Indoor	Lab	Lab	Activity	72	45.5	Fraker 2009
				Lab	Lab	Activity	72	53.4	
Rana temporaria	Dragonfly	Both	Indoor	Lab	Lab	Activity	72	35.6[c]	This study
				Natural	Lab	Activity	72	37.0	

[a]Measured at two temperatures.
[b]No estimate possible: response did not decay over 6 h.
[c]Averaged over the two tadpole treatments.

(indoors/outdoors) had no influence on cue half-life (profile confidence intervals overlapped zero, even at the 70% level). Cue source could not be tested because in this limited dataset it is almost completely confounded with the duration of the experiment. Half-life was estimated to be slightly, but not significantly, longer when cues were aged in tap water instead of natural water (1.6-fold longer; 95% CI: 0.88–3.21 fold). This agrees with the proposition that biodegradation occurs (Beklioglu et al. 2006; Peacor 2006), but the magnitude of the effect is tiny compared with the >600-fold variation in half-life observed among studies. Indeed, visual examination of the data confirms that study duration is the only effective predictor of cue persistence (Fig. 2).

These results raise the question of why the rate of decay in behavioral response to a predator cue depends so strongly on the length of the study. One possibility is that study design drives the outcome: Short studies cannot produce long estimates of cue persistence. This explanation is probably not correct. Indeed, some studies estimated half-lives considerably longer than their duration. Also, most of the shorter studies appeared to be appropriately designed, with nearly complete disappearance of the behavioral response even within a few hours (Fig. 2). Another possibility is that investigators plan the duration of their experiment with some prior information about cue persistence based on preliminary data or natural history knowledge of the study system. Chivers et al.

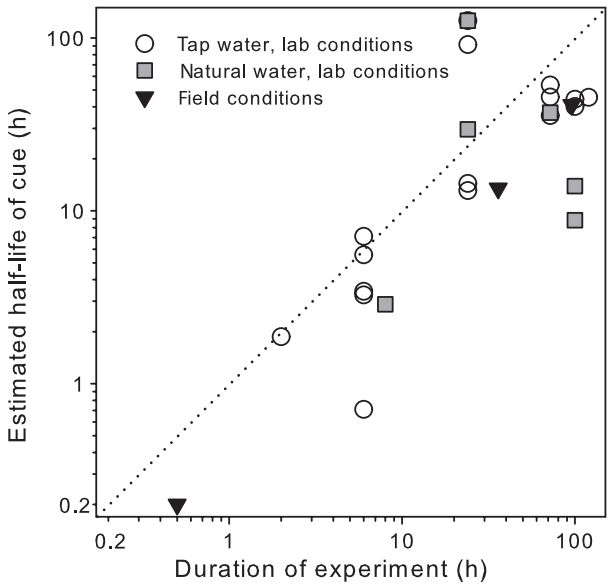

Fig. 2: Results of studies measuring degradation of water-borne chemical cues indicating predation risk (Table 2). The dotted line indicates the situation in which the estimated half-life equals the duration of the study. Analysis of these data indicated that studies of longer duration observed more persistent cues, but there were no effects of the aging venue, the type of aging water, the observation venue, or the behavioral trait used to gauge the response.

(2013) reported that they performed a pilot study to determine the appropriate timeframe and dosage, and this was also true in our case: we based the 72-h duration of our study on a variety of earlier experiments in our laboratory suggesting that odonate cues are effective both indoors and outdoors for about two days. This explanation may account for variation in experimental designs, but it leaves unanswered the question of why the persistence of predator cues varies over >2 orders of magnitude in different systems. This is clearly an area where it would be helpful to understand the chemical composition of predator cues and the mechanisms underlying their degradation (Ferrari et al. 2010).

Our results provide insight into the temporal and spatial dynamics of interactions between predators and prey in aquatic systems. Laundre et al. (2001) envisioned spatial and temporal variation in risk assessment as a 'landscape of fear' experienced by prey, in which hills and valleys correspond to areas of high and low perceived risk. A rugged landscape of fear reflects heterogeneity in the potential strength of lethal and non-lethal interactions and influences the distributions of prey and their resources at lower trophic levels (Valeix et al. 2009; Matassa & Trussell 2011). What is the configuration of the landscape of fear in the aquatic systems summarized in Table 1? Turner & Montgomery (2003) suggested that prey will perceive uneven degrees of risk—that is, the landscape of fear will have 'relief'—when the following inequality is fulfilled: $V\ t\ 2d < (1/n)$, where V is the movement speed of the predator, t is the active lifetime of the cue, d is the distance over which the cue is active, and n is the density of predators. Field studies in freshwater ponds suggest that predator cues trigger behavioral reactions in prey over a range of 1–2 m (Turner & Montgomery 2003; Wisenden 2008). For predators that are common and move rapidly, such as *Lepomis* sunfish (0.01–0.1 fish/m^2; 100 m/h; Mittelbach 1981; Turner & Montgomery 2003), prey will not perceive spatial or temporal variation in predation risk unless the cue has an active lifetime of under 3–30 min. For larval aeshnid predators, which usually move in the range of 1 m/h or less and occur frequently at densities up to 10/m^2 (Van Buskirk 1992; Van Buskirk 2009), the active lifetime of the cue would have to be <10 min. The estimates of cue half-life in Table 2 are greater than this. Conservatively equating half-life with the active lifetime of the chemical, this implies that prey are nearly always exposed to a chemical environment indicating risk. In wetlands where insect predators are scarce, for example 0.1/m^2, the landscape of perceived risk may become uneven if the active lifetime of the cue is below 5 h. In this range of cue persistence, there is disagreement among the published studies in Table 2, and estimates from different organisms and contexts would be helpful. These calculations assume that chemical signals originate only from the predator; the landscape of fear will be more rugged if alarm signals from the prey are also necessary and if predators capture prey infrequently.

Theory predicts that the reliability of the cue indicating the state of the environment strongly affects the evolution of phenotypic plasticity (Moran 1992; Tufto 2000; Donaldson-Matasci et al. 2013). The reliability of a predator cue depends on the temporal and spatial scales of cue attenuation relative to the rate at which predation risk itself changes in time and space. Gabriel et al. (2005) and Fraker (2009) have argued that, if chemical cues linger long after predators depart, prey relying on chemical signals will overestimate risk and therefore suffer recovery lags after predators depart. Indeed, inaccurate assessment of the environment can be detrimental, but adaptive trait expression tends to be conservative with respect to predation risk because it is so costly to decide prematurely that a dangerous environment is predator free. Individual prey often remember predators after they have departed and continue to express antipredator

phenotypes even when there is no immediate indication of risk (Van Buskirk 2002b; Ferrari et al. 2010). The potential for risk overestimation also depends to some extent on the lability of the antipredator phenotype. If traits can be readily altered to match current conditions, persistent cues would indeed lead to overestimation of risk and overexpression of defensive phenotypes. But for traits that develop slowly and are not easily reversed, a persistent cue may better reflect long-term risk and prove to be a reliable indicator that investment in antipredator traits should begin. These two kinds of traits correspond to behavioral and morphological defenses, respectively, in many organisms (West-Eberhard 1989) including anuran larvae (Van Buskirk 2002b; Relyea 2003). We therefore suspect that prey do not usually overreact to predators, even when they exploit a persistent chemical signal indicating risk.

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